

U.S.S.N. 10/521,920

Final Office Action mailed January 23, 2008 (additional comment mailed March 28, 2008)

Amendment under 37 C.F.R. 1.116 filed May 19, 2008

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## REMARKS

Applicants have amended the claims to a preferred embodiment. Namely, claim 1 has been amended to specify that the cells maintain the function to metabolize xenobiotics or cytochrome P450 gene expression. As explained at paragraphs [0014] – [0016], in the area of liver toxicology, there can be a significant lag between exposure to a compound and its manifestation of toxicity. Being able to culture primary hepatocytes that have these functions permits one to determine if a compound becomes toxic over time. However, prior to this invention, such function could not be maintained in culture over long time periods. Support for this amendment can be found throughout the specification, and for example in paragraph [0039] of the specification. Claims 2, 4, and 6-8 have been amended to clarify the antecedent basis for the terms as shown, *supra*. These amendments are clerical. Accordingly, the entry of the amendments is respectfully requested.

Applicants have added new claims 9-15. Support for the new claims can be found, for example in paragraphs [0015], [0024], [0025], [0028], [0056], and [0087]. Accordingly, the entry of the new claims is respectfully requested.

Applicants now turn to the specific rejections.

In the January 23, 2008 Office Action, the Examiner rejected claims 1-8 as allegedly not complying with 35 U.S.C. §112, second paragraph definiteness requirement. This rejection was identical to the one presented in the previous Office Action. Applicants contacted the Examiner. The Examiner acknowledged, as stated in the March 28, 2008 Office Communication, that this rejection was inadvertently left in the Office Action although the Amendment dated November 15, 2007 had overcome the rejection. Accordingly, Applicants respectfully submit that claim 1-8 fully comply with 35 U.S.C. §112, second paragraph definiteness requirement.

The Examiner rejected claims 1, 2, and 4 under 35 U.S.C. §102(b) as allegedly anticipated over U.S. Patent No. 5,198,432 to Fariss (“Fariss”) in view of ATCC catalogue and the Sharma et al. 1994 publication (“Sharma”). The Examiner contended that the ‘432 patent “discloses a method for plating and culturing primary hepatocytes in Waymouth’s medium supplemented with antioxidant vitamin E including tocopherol succinate for 5-7 days on collagen surfaces.” The Examiner further asserted that Fariss “clearly states that the hepatocytes remain viable for 5-7 days.”

Applicants respectfully disagree and submit that the rejection be withdrawn for the following reasons.

Fariss is directed to methods for protection of hepatocytes that have been exposed to toxins. Accordingly, toxins are added to the cells and then various compounds are being tested for their use as a protective agent.

Fariss describes culture of rat hepatocytes **in suspension culture** (col. 6, lines 33-54). Nothing in Fariss describes benefits obtained for the life span of **normal plated primary hepatocytes** by adding any agents to them. The passage in col. 14, lines 20-60 of the '432 patent specifically describes culture conditions for plated cells as a "negative control" (normal culture medium), "positive control" (EMS only, i.e. the toxin) and "EMS + d1-alpha-TP." The experiment was run for only 6 hours, i.e. the cells in the "EMS + d1-alpha-TP" column were exposed to the combination of these two agents for 6 hours. There is no description of cell culture conditions, wherein the cells are grown with or without toxin exposure and in the presence of d1-alpha-TP for more than 6 hrs. The reference to hepatocytes having a life span of 5-7 days, refers to cells that are grown on a collagen matrix in general, as opposed to suspension cultures. There is nothing in the description that indicates that these normal collagen-matrix grown cells have been exposed to d1-alpha-TP. Thus, this adds nothing to what is already described in the background of the present application, which is that the life span of normal primary cultured hepatocytes is about 3-7 days (see, par. [005]).

There is also nothing in Fariss that suggests that the hepatocytes maintain the claimed functions for at least 5 days. By contrast, the present claims require that the primary hepatocyte cultures are grown for at least 5 days in the presence of an anti-oxidant(s) and a second agent, wherein said second agent is (1) a functional inhibitor of an enzyme that generates reactive oxygen and reactive nitrogen species, (2) an agent that directly inhibits the reactive oxygen or the reactive nitrogen species, or (3) an agent that increases intracellular glutathione. Sharma only described use of rat liver cell line (BRL3A) cells in a GSH assay. Applicants wish to point out that these cells are **not primary hepatocytes**, but an **immortalized cell line**. Accordingly, extrapolation of the response of these cells to any test agents, including folic acid, is not necessarily directly applicable to primary hepatocytes.

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Moreover, the new claims are directed to embodiments wherein the claimed primary hepatocyte cultures are **subsequently exposed** to a candidate compound, such as a drug candidate, for more than 3 days. Such claims are certainly not anticipated by the cited prior art.

Accordingly, Applicants respectfully submit that the rejection of claims 1, 2, and 4 under 35 U.S.C. §102(b) as allegedly anticipated over Fariss in view of ATCC catalogue and the Sharma should be withdrawn.

The Examiner rejected claims 1-8 under 35 U.S.C. §103(a) as allegedly obvious over Fariss, the ATCC catalogue and the 1994 Sharma publication further in view of Pourahmad, Dilworth, and Roberts cited in the IDS.

Applicants respectfully disagree and submit that the rejection be withdrawn for the following reasons.

As described, *supra*, Fariss does not disclose the method of the present invention because it does not describe plating and culturing non-toxin exposed primary hepatocytes with any antioxidant or a second agent that is (1) a functional inhibitor of an enzyme that generates reactive oxygen and reactive nitrogen species, (2) an agent that directly inhibits the reactive oxygen or the reactive nitrogen species, or (3) an agent that increases intracellular glutathione for **at least 5 days**. Maximum exposure time to the combination of toxin and d1-alpha-TP is 6 hours.

Neither Pourahmad, Dilworth, nor Roberts overcome this deficiency. All that the references describe is effects of various agents belonging to the genus of the “second agent” of the claims on primary hepatocyte cultures for significantly less than 5 days as already discussed in the Amendment dated November 15, 2007.

Accordingly, Applicants respectfully submit that the rejection of claims 1-8 under 35 U.S.C. §103(a) as allegedly obvious over Fariss, the ATCC catalogue and the 1994 Sharma publication further in view of Pourahmad, Dilworth, and Roberts should be withdrawn.

In view of the foregoing, Applicants respectfully submit that all claims are in condition for allowance. Early and favorable action is requested.

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The Commissioner is herewith authorized to charge fee deficiencies and credit overpayments to the NIXON PEABODY LLP Deposit Account No. 50-0850.

Date: May 19, 2008

Respectfully submitted,

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